Please amend the claims as follows:

7. (Amended) The method of claim 1, wherein the immune response reduces or inhibits growth of the pathogen.

24. (Twice amended) A pharmaceutical composition comprising a tumor antigen RNA and a pharmaceutical carrier, which pharmaceutical carrier is suitable for *in vivo* delivery to a human.

REMARKS

Claims 1-31 are pending in the application. Claims 24 has been amended to place the claim in better form for allowance. Claim 7 has been amended to correct an inadvertent typographical error. No new matter is introduced with these changes. For convenience, a copy of the pending claims as amended is attached.

Rejection under 35 U.S.C. §112

Claims 1-30 stand rejected under 35 U.S.C.§ 112 first paragraph. The Examiner asserts that the specification does not provide enablement for protecting a subject from or inducing tolerance in a subject to the variety of "antigens" and the various modes of administration encompassed by the scope of the claims. Applicant respectfully traverses the rejection.

As applicant has asserted in the previous response and as the Examiner has acknowledged in the Office Action, the methods and materials for practicing the claimed invention are fully supported in the specification. In addition, contrary to the Examiner's assertion that the claimed invention is not enabled because of the unpredictable outcome of the invention as broadly claimed, applicant asserts that the specification, when considered with the teachings of the state of the art at the time of the invention, enables the full scope of the claimed invention both in the area of induction of protective immunity and in the area of induction of tolerance for the antigens encompassed by the scope of the claims.

Contrary to the assertion by the Examiner that RNA vaccination,

especially intravenous administration, does not protect a subject, much literature at the time of the invention reported that genetic immunization with DNA by various modes of administration resulted in potent activation of immune responses. For example, administration into muscle or skin by needle injection as well as delivery by gene gun into the skin has been studied. It has been demonstrated that intradermal injection is more efficient than intramuscular injection for establishing humoral immune responses (Tang et al., *Nature*, 1992, 356:152-154; Exhibit 1). Although it has also been shown that administration by gene gun may require less DNA than other methods, the state of the art at the time of the invention was that successful DNA vaccination may be obtained by a variety of routes of administration (Fynan et al., *Proc. Natl. Acad. Sci. USA*, 1993, 90:11478-11482; Exhibit 2).

> "The breadth of routes supporting successful DNA immunizations, coupled with the very small amounts of DNA required for gene-gun immunizations, highlight the potential use of this remarkably simple technique for the development of . . . vaccines.
> (Exhibit 2, page 11478)

Thus the art shows that, as pointed out by the Examiner, the routes of administration of a DNA vaccine may influence the outcome. However, the method and route of administration may affect the <u>degree</u> of immunity engendered, not that an immune response could not reasonably be predicted by one of skill in the art. That the administration of DNA elicits an immune response is clearly established by the state of the art at the time of the invention.

With regard to tumor immunity, much literature at the time of the invention has shown induction of potent immunity with DNA-based vaccines. For example, immunization of mice with DNA coding for human chorionic gonadotropin β (hCGβ) subunit by the intramuscular route significantly inhibited the growth of a myeloma expressing HCGβ (Geissler et al., *Lab. Invest.*, 1997, 76:859-871; Exhibit 3). In another study, intramuscular administration of DNA coding for the paraneoplastic encephalomyelitis antigen HuD provided significant protection against

subsequent challenge with HuD expressing tumor (Ohwada et al., *Am. J. Respir. Cell Mol. Biol.*, 1999, 21:37-43; Exhibit 4). In another example, mice immunized by intramuscular injection with a cDNA for prostate specific antigen (PSA) produced a strong antibody and cytotoxic lymphocyte response against tumor cell targets expressing PSA (Kim et al., *Oncogene*, 1998, 17:3125-3135; Exhibit 5). Intramuscular immunization of mice with a cDNA encoding the ovarian carcinoma-associated antigen folate receptor α induced both cytotoxic T-lymphocyte and antibody responses in mice (Neglia et al., *Cancer Gene Ther.*, 1999, 6:349-357; Exhibit 6). Vaccination significantly inhibited growth of subsequently administered folate receptor α expressing tumor cells. Critically, immunization in this manner after intravenous injection of tumor cells enhanced the mean survival time and reduced the number of lung metastases, showing the applicability of genetic immunization to pre-existing tumor cells.

Dendritic cell immunization has also been shown in the literature at the time of the invention to provide protection against established tumors. For example, immunization of mice with dendritic cells transduced with an adenovirus vector encoding the human gp 100 melanoma antigen resulted in the generation of potent cytotoxic lymphocytes against gp100 expressing cells (Kaplan et al., *J. Immunol.*, 1999, 163:699-707; Exhibit 7). Immunization was also associated with long-term protection against lethal challenge with B16 melanoma cells. Immunization of mice with dendritic cells transduced with tyrosine related protein-2 also induced a protective response. Significantly, dendritic cell based immunization induced partial protection against established B16 tumors. The degree of protection seen was improved by simultaneous immunization with both melanoma associated antigens compared to immunization with either alone.

With respect to intradermal vaccination, a study of the immune response to a hepatitis B DNA vaccine in Aotus monkeys showed an enhanced humoral response by intradermal administration (Gramzinski et al., *Mol. Medicine*, 1998,

4:109-118; Exhibit 8).

With respect to dendritic cells transduced with tumor RNA, the effectiveness of this method in generating anti-tumor immunity is disclosed in the present application.

With regard to the use of genetic immunization for immunity against microbial antigens, the efficacy of a DNA vaccine has been demonstrated against influenza (Ulmer et al., *Science*, 1993, 259:1745-1749; Exhibit 9). In this study it was shown that immunization with influenza A nucleoprotein produced a specific cellular immune response and protection from challenge with influenza strains. It has also been shown in the literature at the time of the invention that:

- Mice immunized with a DNA vaccine against Sendai virus demonstrated long-lasting cytotoxic lymphocyte activity; upon challenge one year after immunization, viral titers in the lungs of challenged mice were 100-fold less in immunized animals compared to control animals (Chen et al., *J. Gen. Virol.*, 1999, 80:1393-1399; Exhibit 10).
- Immunization of mice using DNA encoding *Listeria listeriolysin* O by intramuscular administration demonstrated potent cytotoxic activity and protection against subsequent challenge with viable *Listeria monocyotogenes* (Cornell et al., *J. Immunol.*, 1999, 163:322-329; Exhibit 11).
- Immunization of mice with a plasmid containing DNA for several mycobacterial antigens together with a cDNA for green fluorescent protein resulted in significant protection against subsequent infection with *Mycobacterium avium* (Velaz-Faircloth et al., *Infect. Immun.*, 1999, 67:4243-4250; Exhibit 12).
- Mice immunized with a replicon encoding *Heliobacter pylori* urease demonstrated a significant T helper 1 type immune response (Novak et al., *Vaccine*, 1999, 17:2384-2391; Exhibit 13).
- Protection of turkeys against *Chlamydia psittaci* was demonstrated after cutaneous immunization with DNA expressing the major outer membrane

protein of avian *Chlamydia psittaci* via gene gun (Vanrompay et al., *Vaccine*, 1999, 17:2628-2635; Exhibit 14).

In addition, submitted herewith is the Declaration under Rule 132 of Dr. Richard Granstein which presents data demonstrating that the claimed invention is effective in reducing the rate of tumor growth in two mouse tumor models.

The foregoing demonstrates that the combination of the state of the art at the time of the invention and the teachings in the specification provide enablement for protecting a subject from a variety of "antigens" and the various modes of administration encompassed by the scope of the claims.

The claimed invention is also fully enabled for the asserted property of inducing tolerance. Applicant has demonstrated that the claimed invention induces tolerance by intravenous administration of RNA. As shown in the data in the specification, intravenous administration of total cellular RNA from the S1509a tumor cell line induces tolerance with inhibition of the ability to immunize to that tumor.

In addition, applicant provided in the response filed September 20, 2001 the Declaration under Rule 132 of Richard D. Granstein, the named inventor of the above-captioned application, that demonstrates that the claimed invention, i.e., tolerization, is achieved and therefore fully enabled. The experiment described in the Declaration demonstrates that tolerance is induced by intravenous administration of total cellular RNA from S1509a tumor cells. The results also show that tolerance can be adoptively transferred to naive recipients by transfer by splenic lymphoid cells. Furthermore, antibody and complement-mediated deletion of T cells from the transferred population prevents transfer of tolerance.

Also, the literature available at the time of the invention documents that administration of protein antigens, including alloantigens, by the intravenous route induces immunologic tolerance. In addition to induction of tolerance to tumor antigens and alloantigens by the intravenous route, there is considerable evidence that intravenous administration of microbial antigens also will induce tolerance. For

example, Shepard and colleagues showed that intravenous injection of killed *Mycobacterium leprae* into mice induced immunologic tolerance (Shepard et al., *Infect. Immun.*, 1982, 38:673-680; Exhibit 15). Similarly, injection of lipopolysaccharide, a component of gram negative bacteria cell membranes, resulted in reduction in lung clearance of *Pseudomonas aeruginosa* after aerosol challenge (Mason et al., *J. Infect. Dis.*, 1997, 176:1293-1302; Exhibit 16). Intravenous administration of dead *Mycobacterium leprae* into rats induced specific immunologic tolerance (Winters and Humphres, *Infect. Immun.*, 1990, 58:495-501; Exhibit 17).

The foregoing demonstrates that the combination of the state of the art at the time of the invention and the teachings in the specification provide enablement for inducing tolerance in a subject from a variety of "antigens" and the various modes of administration encompassed by the scope of the claims.

While intravenous administration of RNA for induction of tolerance has not previously been reported, that merely demonstrates the novelty of the claimed invention.

All of the foregoing demonstrate with credible scientific evidence that all of the teachings needed to practice the claimed invention were well within the knowledge of those skilled in the art.

The first paragraph of Section 112 requires nothing more than objective enablement. *In re Marzocchi and Horton*, 169 USPQ 367, 369 (CCPA 1971). A rejection for failure to teach how to make and/or use the claimed invention can be overcome by suitable proofs indicating that the teaching contained in the specification is truly enabling. *Id*. Such proofs may be in the form of pertinent references that show the state of the art at the time of the invention:

> Most often, additional factors, such as the teachings in pertinent references, will be available to substantiate any doubts that the asserted scope of objective enablement is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof.

In re Marzocchi and Horton, 169 USPQ 367, 370 (CCPA 1971)

Applicant has met the requirements of Section 112. Applicant has provided credible scientific proof in the form of literature available at the time of the invention and the Declaration of Dr. Granstein which demonstrate that the combination of the specification and the state of the art at the time of the invention fully enable the full scope of the claimed invention. The present specification enables one of skill how to use the claimed invention and provides a reasonable degree of assurance of success in practicing the full scope of the claimed invention.

Thus, in view of the arguments of record and the evidence presented herein, applicant submits that the specification provides full enabling support for the claimed invention. Applicant respectfully requests that the Section 112 rejection be withdrawn.

Rejection under 35 U.S.C. §102

Claims 24 and 30 stand rejected as anticipated by Qiu et al. Applicant respectfully traverses the rejection.

Qui does not disclose the claimed invention because the compositions of Qui do not meet the standards required for in vivo delivery into humans required by the present invention. As discussed above, applicant sets the standard in the specification for pharmaceutical carriers that are suitable for the invention as those carriers approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans." (see page 20, lines10-13). Instead, Qui utilized RNA/gold particle complexes.

In addition, in order to place the claims in better form for allowance, claim 24 has been amended to recite "a tumor antigen RNA".

In view of the foregoing comments and amendments to the claims, applicant respectfully requests that the Section 102 rejection in view of Qui be withdrawn.

CONCLUSION

In light of all of the foregoing, reconsideration and withdrawal of the rejections are respectfully requested. Applicant submits that all of claims 1-31 are in condition for allowance. Prompt and favorable allowance of the claims is respectfully requested.

Respectfully submitted,

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PENDING CLAIMS

including the amendments in the Response to Final dated April 23, 2002

1. A method of inducing an immune response to a pathogen comprising administering to epidermal cells of a recipient total pathogen cell RNA in an amount effective to elicit an immune response against the pathogen.

2. The method of claim 1 wherein the total cell RNA is administered to the epidermal cells *in vitro*.

3. The method of claim 2 wherein the epidermal cells are modified by pulsing the cells with the total RNA.

4. The method of claim 1 wherein the total cell RNA is administered directly into the epidermal cells of the recipient *in vivo*.

5. The method of claim 1, wherein the pathogen is a tumor.

6. The method of claim 5, wherein the tumor is a fibrosarcoma tumor.

7. The method of claim 1, wherein the immune response reduces or inhibits growth of the pathogen.

8. A pharmaceutical composition comprising total pathogen cell RNA and a pharmaceutical carrier, which pharmaceutical carrier is suitable for *in vivo* delivery to a human.

9. The composition of claim 8, wherein the pathogen is a tumor.

10. The composition of claim 9, wherein the tumor is a fibrosarcoma tumor.

11. A method for protecting a subject from a cancer which method comprises delivering an immunologically effective amount of total tumor cell RNA to the subject, wherein the tumor cell is of the type associated with the cancer.

12. The method of claim 11, further comprising delivering an immunostimulatory amount of an immune activating or inflammatory cytokine to the subject.

13. A vaccine comprising a pathogen total cell RNA and an adjuvant acceptable for use in a human.

14. The vaccine of claim 13, wherein the pathogen is a tumor.

15. The vaccine of claim 14, wherein the tumor is a fibrosarcoma tumor.

16. A method of inducing immune tolerance to an antigen, which method comprises administering antigen RNA in an amount effective to elicit immune tolerance against the antigen.

17. The method of claim 16, wherein the RNA is total cellular RNA from tissues containing the antigen.

18. The method of claim 16, wherein the RNA is total cellular mRNA from tissues containing the antigen.

19. The method of claim 16, wherein the RNA is mRNA encoding the antigen.

20. The method of claim 16, wherein the RNA is administered intravenously, orally, or intranasally.

21. The method of claim 16, wherein the antigen is an autoantigen.

22. The method of claim 16, wherein the antigen is an allergen.

23. The method of claim 16, wherein the antigen is a transplant antigen.

24. A pharmaceutical composition comprising a tumor antigen RNA and a pharmaceutical carrier, which pharmaceutical carrier is suitable for *in vivo* delivery to a human.

25. The composition of claim 24, wherein the antigen is an autoantigen.

26. The composition of claim 24, wherein the antigen is an allergen.

27. The composition of claim 24, wherein the antigen is a transplant antigen.

28. The composition of claim 24, wherein the RNA is total cellular RNA from tissues containing the antigen.

29. The composition of claim 24, wherein the RNA is total cellular mRNA from tissues containing the antigen.

30. The composition of claim 24, wherein the RNA is mRNA encoding the antigen.

31. A method for protecting a subject from a cancer which method comprises delivering to epidermal cells of a subject an immunologically effective amount of total tumor cell RNA, wherein the tumor cell is of the type associated with the cancer.